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NEUROLOGICAL UPDATE

# Non-invasive biomarkers for diagnosis and monitoring of primary mitochondrial diseases

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## Abstract

Primary mitochondrial diseases (PMDs) represent a clinically and genetically heterogeneous group of disorders characterized by impaired oxidative phosphorylation and multisystem involvement, commonly affecting the nervous system. As therapeutic development accelerates, there is a growing need for robust biomarkers capable of supporting diagnosis, stratifying patient subgroups, monitoring disease progression, and providing sensitive pharmacodynamic readouts for clinical trials. This review summarizes recent advances in three major non-invasive biomarker domains relevant to PMDs: circulating serum and molecular biomarkers, functional and digital endpoints, and neuroimaging modalities. Circulating markers, such as FGF21, GDF15, NfL, and $NAD^+$-related signatures, have each been proposed for diagnosis and to follow disease progression, while multi-omics approaches are paving the way toward integrated molecular phenotyping. Digital health technologies, including accelerometry and gait analytics, enable objective quantification of real-world functional impairment, although disease-specific validation remains an unmet need. Neuroimaging offers mechanistic insights through metabolic (MRS, CEST), perfusion (ASL), and molecular modalities (mitochondrial PET tracers). Cutting-edge tools, such as Multi-Spectral Optoacoustic Tomography (MSOT), Raman spectroscopy, and Near-Infrared Spectroscopy (NIRS), promise real-time or spatially resolved assessment of mitochondrial function. Together, these developments outline multidimensional biomarker approaches for PMDs, with the potential to directly measure target engagement and clinically meaningful phenotypes in future therapeutic trials. Future progress will depend on longitudinal validation, harmonized acquisition protocols, and the integration of multimodal platforms to support upcoming therapeutic trials and precision medicine strategies.

**Keywords** Mitochondrial disease · Biomarkers · Functional endpoints · Wearable devices · Digital health technologies · Phenotyping · Neuroimaging · Magnetic resonance imaging · Positron emission tomography · Precision medicine · Clinical trials

## Introduction

Primary mitochondrial diseases (PMDs) comprise a clinically heterogeneous group of disorders caused by pathogenic variants in mitochondrial or nuclear genes, resulting in impaired oxidative phosphorylation (OXPHOS). With an estimated prevalence of approximately 1 in 4,300 individuals [1], PMDs represent one of the most common inherited metabolic disorders. They may present with a wide range of neurological and multisystem manifestations, each posing significant challenges for diagnosis and management [2].

Despite major advances in genetic diagnosis [3] and the understanding of mitochondrial disease pathophysiology [4, 5], effective therapeutic options remain limited [6]. To date, only three drugs have received regulatory approval for the treatment of specific forms of PMDs: idebenone for Leber's Hereditary Optic Neuropathy (LHON) [7] in Europe, and more recently, elamipretide for Barth Syndrome [8] and deoxy-pyrimidines for Thymidine kinase 2 (TK2)-related mtDNA depletion syndrome [9, 10] in the United States. However, most mitochondrial syndromes still lack

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disease-modifying interventions [6, 10–13], and an increasing number of emerging therapeutic strategies underscore the urgent need for reliable and clinically meaningful biomarkers [14, 15].

Biomarkers, defined as measurable indicators of normal biological processes, pathogenic mechanisms, or responses to therapeutic interventions [16], hold particular promise in PMDs. Given the pronounced clinical heterogeneity and diagnostic complexity of these disorders, robust biomarkers with direct relevance for the underlying pathophysiology are essential to improve diagnostic accuracy and patient stratification. There is an urgent need for biomarkers to monitor disease progression, predicting acute neurological events such as stroke-like episodes or sudden visual loss, and objectively assessing treatment responses or target engagement in experimental medicine studies and clinical trials [17].

Recent years have witnessed progress in biomarker discovery, driven by targeted and untargeted omics approaches, advances in imaging, and in digital and functional assessments tailored to specific phenotypes [18]. However, despite their promise, most still require rigorous validation.

The translation of candidate biomarkers into clinical practice or trials requires a progressive stepwise process of analytical and clinical validation followed by regulatory qualification within frameworks established by agencies, such as the European Medicines Agency (EMA), the U.S. Food and Drug Administration (FDA), or the UK Medicines and Healthcare products Regulatory Agency (MHRA). Biomarkers must demonstrate robust validity, including reproducibility, sensitivity and specificity and must be clearly defined within a specific "context of use" (e.g., diagnostic, monitoring, pharmacodynamic (response), predictive, etc.)[19]. This process typically requires evidence derived from multiple independent cohorts, standardized analytical methods, and harmonized protocols across centers. These challenges are amplified in rare diseases such as PMDs due to limited patient populations and heterogeneous disease progression [20].

Consequently, trial outcome measures have largely relied on established generic clinical or functional endpoints, rather than biomarkers that provide direct and short-term measures of target engagement or mitochondrial function. For example, studies on elamipretide in Barth Syndrome relied on echocardiography; idebenone in LHON stabilized and restored visual acuity; and deoxynucleoside supplementation in TK2-deficiency improved respiratory function, the 6-min walk test and reduced GDF15 levels [7–9].

This review provides an overview of the most relevant current and emerging clinical biomarkers for PMDs, highlighting their applicability and future utility for longitudinal disease monitoring. We summarize advances in laboratory biomarkers, neuroimaging modalities, and digital endpoints, and discuss possible future directions, such as molecular imaging and integrative multi-omics (Fig. 1, Table 1). As novel therapies advance toward clinical translation, the development and validation of sensitive, specific, and reproducible biomarkers will be critical to enable early diagnosis, optimize patient stratification, and reliably evaluate therapeutic efficacy.

## Functional assessments: from motor tests to digital monitoring

Fatigue is one of the most prevalent and disabling symptoms reported by individuals with PMDs, profoundly affecting daily functioning and quality of life and frequently co-occurring with muscle weakness, pain, and reduced mobility [21]. Consequently, the evaluation of fatigue and motor performance is central to both clinical monitoring and assessment of therapeutic efficacy.

Traditional functional assessments, including manual muscle testing, functional motor scales, cardiopulmonary exercise test and timed performance measures, have been adapted from other neuromuscular diseases and validated in PMDs [22–26]. However, these tools are often limited by inter-rater variability, ceiling and floor effects, motivational dependence, and insufficient sensitivity to capture subclinical or slowly progressive changes, particularly in heterogeneous and multisystem disorders [27].

Digital health technologies (DHTs) have emerged as a promising avenue to overcome these limitations. Wearable and non-wearable sensors enable objective and continuous quantification of motor and physiological function, either during structured in-clinic assessments or remotely during everyday activities [28]. Their successful implementation in other neuromuscular diseases, such as Duchenne muscular dystrophy, where digital gait biomarkers (e.g., Stride Velocity 95th Centile) have been qualified by regulatory agencies, provides a compelling precedent for their potential application in PMDs [29].

Initial investigations in PMDs indicate that wearable-based assessments are both feasible and informative. One of the earliest cross-sectional studies employing actigraphy demonstrated significantly reduced habitual physical activity in PMD patients, with daily step count inversely correlating with disease burden measured by the Newcastle Mitochondrial Disease Adult Scale (NMDAS) [30]. Complementary work using portable gait analysis systems revealed discrete gait abnormalities in adults with m.3243A > G and m.8344A > G variants, even among mildly affected individuals, underscoring the sensitivity of accelerometry-based tools to detect subtle motor impairments [31]. In pediatric cohorts, accelerometry similarly distinguished affected children from age- and sex-matched controls across domains of physical activity [32]. More

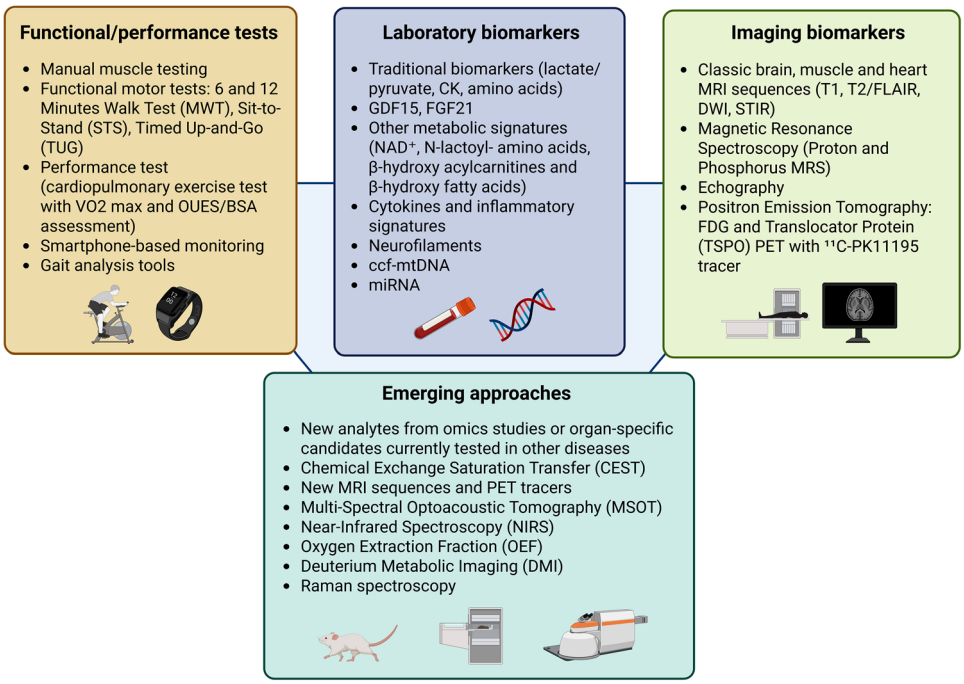


**Fig. 1** Non-invasive monitoring in primary mitochondrial disorders (PMDs). Biomarkers and tests for three major non-invasive monitoring domains in PMDs include functional and performance motor tests, laboratory biomarkers, and imaging biomarkers. Emerging approaches include promising examples of biomarkers that have been tested in model systems or patients with other neurodegenerative or neuromuscular conditions with potential future applications for PMDs though further studies are still needed. *DWI* Diffusion-Weighted Imaging, *FDG* Fluorodeoxyglucose, *FGF21* Fibroblast Growth Factor 21, *GDF15* Growth Differentiation Factor 15, *MRI* Magnetic Resonance Imaging, *MRS* Magnetic Resonance Spectroscopy, *OUES/BSA* Oxygen Uptake Efficiency Slope/Body surface Area, *PET* Positron Emission Tomography, *VO2* max Maximal Oxygen Consumption

advanced gait quantification platforms have also shown excellent reproducibility. The GAITRite electronic walkway has been validated in adults with the m.3243A > G variant, demonstrating high intra-class correlation coefficients across walking conditions and significant correlations between gait metrics and NMDAS scores [33]. Nonetheless, the responsiveness of wearable-derived outcomes remains an area of active investigation: in a longitudinal study assessing bezafibrate treatment, no significant change was observed in accelerometry-based activity levels, despite biochemical improvement, illustrating both the promise and challenges of incorporating digital endpoints into PMD trials [34].

Collectively, wearable and portable technologies can provide objective, reproducible, and clinically meaningful assessments of motor function and physical activity in PMDs. To advance their integration into clinical research, large-scale longitudinal studies, harmonized protocols, and disease-specific validation frameworks are needed. Future work should aim to develop multimodal digital biomarker profiles—combining movement data with cardiorespiratory, sleep, and fatigue-related metrics—to comprehensively capture the complex and fluctuating functional impairments that characterize PMDs.

## Laboratory biomarkers: untargeted and targeted approaches

Given the predominant bioenergetic impairment and frequent muscle involvement in PMDs, traditional CSF and plasma analytes, such as lactate, lactate/pyruvate ratio, amino acids, and creatine kinase (CK), have long served as adjunct biochemical indicators to support diagnosis, alongside muscle biopsy [35–37]. However, extensive clinical experience has demonstrated limited sensitivity and specificity for both diagnostic and longitudinal monitoring, as they may also reflect secondary mitochondrial dysfunction in a range of conditions including hypoxia, sepsis, seizures, or intense exercise [38, 39]. Therefore, their diagnostic utility has further declined with the widespread implementation of next-generation sequencing [3].

Omics-based approaches have gained considerable traction over the past decade, deepening mechanistic understanding of PMD pathophysiology and providing candidates for subsequent targeted evaluation [40]. Early transcriptomic and metabolomic work in mitochondrial disease models has shown profound oxidative stress remodeling converging on the induction of fibroblast growth factor 21 (FGF21), a metabolic cytokine secreted by stressed muscle

**Table 1** Tentative classification of non-invasive biomarkers, according to both their clinical role (diagnostic, monitoring, pharmacodynamic) and their current level of validation

| Clinical application in PMDs | Exploratory | Partially validated | Clinically actionable |
|---|---|---|---|
| Diagnostic | Cardiopulmonary exercise testing with Oxygen Uptake Efficiency Slope/Body Surface Area (OUES/BSA) | | |
| | miRNA; 'omics signatures | Gelsolin; $NAD^+$; CHIT1 | Lactate; Pyruvate; Lactate/Pyruvate ratio; CK; amino acids; citrulline; Thd; dUrd; GDF15; FGF21 |
| | Raman spectroscopy; Near Infrared Spectroscopy (NIRS) with vascular occlusion test | $^{31}$P-MRS; PET-FDG | CT and MRI standard sequences (T2/FLAIR, DWI); $^{1}$H-MRS (with lactate peak) |
| Monitoring | Smartphone and/or wearable-based monitoring tools; SV95C; Cardiopulmonary exercise testing with Oxygen Uptake Efficiency Slope/Body Surface Area (OUES/BSA) | Gait analysis metrics (e.g., GAITrite) | Traditional functional/performance tests (6- and 12-min walk test, 30-s Sit-to-Stand test, Timed Up and Go test, Cardiopulmonary exercise testing with VO2 max) |
| | miRNA; cytokines; 'omics signatures | FGF21; NfL; pNfH; ccf-mtDNA | Nucleosides (dThd, dUrd); GDF15 |
| | MSOT; Near Infrared Spectroscopy (NIRS); Chemical Exchange Saturation Transfer (CEST); Raman spectroscopy | TSPO PET; $^{31}$P-MRS; $^{1}$H-MRS (with lactate peak) | |
| Pharmacodynamic (response biomarker) | SV95C; digital health-based composite endpoints | | Nucleosides (dThd, dUrd); GDF15* |
| | 'omics signatures | | |
| | PET with new mitochondrial tracers ($^{18}$F-BCPP-EF, TPSO); Oxygen Extraction Fraction (OEF); Near Infrared Spectroscopy (NIRS) with vascular occlusion test; MSOT; Raman spectroscopy; MRI with Deuterium Metabolic Imaging | $^{31}$P-MRS | |

"Exploratory" biomarkers refer to early-stage candidates supported primarily by preclinical studies or small clinical cohorts. "Partially validated" biomarkers have shown reproducible associations with disease biology or severity in independent cohorts but lack widespread clinical implementation or regulatory qualification. "Clinically actionable" biomarkers are currently used in clinical diagnostic workflows or patient management

*ccf-mtDNA* Circulating Cell-Free mtDNA; *CK* Creatine kinase; *FGF21* Fibroblast Growth Factor 21; *GDF15* Growth Differentiation Factor 15; *DWI* Diffusion-Weighted Imaging; *FDG* Fluorodeoxyglucose; *FLAIR* Fluid-Attenuated Inversion Recovery; *MRI* Magnetic Resonance Imaging; *MRS* Magnetic Resonance Spectroscopy; *MSOT* Multi-Spectral Optoacoustic Tomography; *NfL* Neurofilament Light Chains; *OEF* Oxygen Extraction Fraction; *PET* Positron Emission Tomography; *SV95C* Stride Velocity 95th Centile; *TSPO* Translocator protein; *VO2 max* Maximal Oxygen Consumption

*In specific diseases (Thymidine Kinase 2 deficiency and Mitochondrial Neurogastrointestinal Encephalopathy)

fibers [41]. FGF21 has since been validated as a biomarker for mitochondrial myopathies, especially those associated with impaired mitochondrial DNA maintenance or translation [42–48]. Nonetheless, FGF21 can also be elevated in other conditions, including liver diseases and other metabolic neuromuscular disorders. Growth differentiation factor 15 (GDF15), another stress-responsive cytokine, has subsequently emerged as a complementary marker, initially as differentially expressed gene in muscle from patients with TK2-deficiency [49] and later also associated with other PMDs [50, 51]. Although its diagnostic specificity is limited by elevations in cancer, inflammation, and other metabolic and neuromuscular conditions [52], GDF15 has demonstrated promise particularly in TK2 deficiency, where it correlates with disease severity and response to nucleoside supplementation [53, 54]. GDF15 detection in saliva might enable minimally invasive monitoring [55], and integrating measurements with plasma gelsolin was shown to improve diagnostic accuracy [56].

Proteomic profiles from a cohort of mitochondrial patient fibroblasts pointed out the expression of 5 proteins (GPX4, ORF4L1, MOXD1, MSRA, and TMED9), as putative novel biomarkers [57]. Hypocitrullinemia was found in MT-ATP6–related conditions, while tyrosine was shown to be elevated in deoxyguanosine kinase deficiency [58–60]. Metabolomic and multi-omics profiling of urine

samples in carriers of the common MELAS m.3243A > G variant [61] or in patients with progressive external ophthalmoplegia [62], have demonstrated systemic molecular alterations, supporting the sensitivity of urinary profiling in PMDs. Metabolomics have also been applied in plasma from Maternally Inherited Diabetes and Deafness (MIDD) patients [63], and more recently, in Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) [64], showing disease-specific patterns of organ involvement and supporting development of integrated multi-omics signatures rather than reliance on single biomarkers for future clinical trials.

Circulating signatures of NADH-driven reductive stress have been associated with disease burden in m.3243A > G, including well-established (lactate, alanine, GDF15, α-hydroxybutyrate) as well as new groups of analytes such *N*-lactoyl-amino acids, β-hydroxy acylcarnitines, and β-hydroxy fatty acids [65]. Reduced $NAD^+$ levels have been proposed as a biomarker with direct relationship to the underlying biochemical OXPHOS defect in PMDs, and are being explored as a therapeutic target, for example in trials of niacin supplementation [42, 66]. Among the other metabolites with direct relevance for the underlying genetic or biochemical defect, thymidine/deoxyuridine levels are well-established as diagnostic biomarkers in MNGIE due to thymidine phosphorylase deficiency [67], where they also act as response biomarkers to measure therapeutic efficiency of liver or bone marrow transplantation [68, 69], and novel advanced therapies [70–72].

Whereas the aforementioned biomarkers broadly reflect primary mitochondrial dysfunction and may reflect tissue-specific disease phenotypes, several other biomarkers are more broadly associated with organ damage and acute disease activity in other conditions. Several of these have also been explored in PMDs. Neurofilament light chain (NfL), widely used in neurodegenerative disorders, has shown promise as a marker of neuroaxonal injury in PMDs in serum and in the cerebrospinal fluid, where it correlates with clinical severity in MELAS [73–77]. Elevated circulating cell-free mitochondrial DNA (ccf-mtDNA) may act as an indicator of mitochondrial stress during acute MELAS episodes [78, 79]. Chitotriosidase (CHIT1), historically used in lysosomal storage diseases, was found to be elevated in PMDs versus other neuromuscular diseases (NMDs) and healthy controls [80]. Finally, recent studies have identified inflammatory activation and upregulation of interferon-stimulated genes in pediatric PMD cohorts [81, 82], and have explored changes in cytokines [83] or circulating microRNAs as possible stable, disease-related indicators in MNGIE [84], MELAS [85], or patients with PMDs and sensorineural hearing loss [86]. At present, most of these remain adjunctive tools for diagnosis, stratification, and trial readiness, pending longitudinal validation. A summary of the main circulating biomarkers that have been tested in patients with mitochondrial disease, is provided in Table 2.

## Neuroimaging for diagnosis and monitoring

Imaging biomarkers provide non-invasive, spatial readouts of tissue bioenergetics of the brain, heart and skeletal muscle, and potentially detect metabolic derangement and structural injury [87–89]. In PMDs, imaging can support diagnosis, map affected organs and pathways, and monitor progression or treatment response as outcome measures in trials. Classic MRI remains key to diagnose Leigh Syndrome with symmetric T2/FLAIR hyper-intensities in basal ganglia, brainstem, and thalamus [90]. Other characteristic MRI patterns affecting deep gray matter nuclei, thalami, brainstem, cerebellum and cerebral white matter frequently guide clinicians toward a PMD and their differential diagnosis [91–94], while CT may sometimes reveal bilateral basal ganglia calcification. Apart from these classical PMDs, it is important to always consider treatable differential diagnoses, that may present with MRI features similar to for example Leigh Syndrome. MRI is also used in the heart to visualize cardiomyopathies and fibrosis [95], which has been reviewed recently elsewhere [96].

Magnetic resonance spectroscopy (MRS) provides non-invasive metabolic information about the biochemical composition of tissues. Proton MRS ($^1$H-MRS) has long been used to measure metabolic state, with elevated cerebral lactate representing one of the first biomarkers of mitochondrial dysfunction, for example in MELAS [97] or Kearns–Sayre syndrome/Pearson syndrome, where lactate is increased in damaged white matter regions; and Leigh syndrome, where lactate peaks are seen in gray and white matter alongside high choline levels [98, 99]. The detection of lactate in the brain is more consistent than in serum, correlates with disease activity in several cohorts and may provide a CNS-based indicator of OXPHOS failure [100–102], but cannot necessarily be distinguished from cellular hypoxia seen in other conditions such as seizures or hypoxic-ischemic injuries [103].

Phosphorus MRS ($^{31}$P-MRS) enables non-invasive assessment of cardiac and skeletal muscle bioenergetics. By quantifying high-energy phosphate metabolites, such as phosphocreatine (PCr), inorganic phosphate (Pi), and ATP, $^{31}$P-MRS allows real-time evaluation of oxidative phosphorylation capacity [104, 105]. Impaired oxidative metabolism typically manifests as prolonged post-exercise PCr recovery [106]. Abnormalities, such as altered phosphate ratios and delayed recovery kinetics, have been demonstrated in patients with mitochondrial myopathies due to mtDNA variants although diagnostic sensitivity is limited [107]. Exercise-based $^{31}$P-MRS provides a quantitative in vivo

**Table 2** List of the main circulating analytes/biomarkers tested in Primary Mitochondrial Diseases (PMDs)

| Organ | Analyte/Biomarker | Mitochondrial condition(s) | Findings and applications/limits | References |
|---|---|---|---|---|
| Blood | Lactate*<br>Pyruvate*; Lactate/pyruvate ratio | Tested in different PMDs | Elevated in different PMDs but overall limited diagnostic sensitivity/specificity. Pyruvate and lactate/pyruvate could be useful as a complementary biomarker in Pyruvate Dehydrogenase and Pyruvate Carboxylase deficiencies | Paredes-Fuentes et al. [39] |
| | Creatine kinase (CK) | Tested in different PMDs | Elevated in myopathic forms, but limited diagnostic sensitivity/specificity, apart from specific contexts (TK2 deficiency) | Debray et al. [36] |
| | Amino acids (alanine*, aspartate) | Tested in different PMDs | Occasionally elevated, especially in early onset forms. Overall low sensitivity and specificity | Paredes-Fuentes et al. [39] |
| | Citrulline | ATP6-related disorders | Reduced in ATP6-related disorders | Li et al., [58]; Carli et al. [59] |
| | Thymidine (Thd), deoxyuridine (dUrd) | MNGIE | Thd and dUrd levels are useful in MNGIE patients | Hirano et al. [67] |
| | FGF21 | Tested in different PMDs, mostly mitochondrial myopathies | Elevated in mtDNA maintenance/translation defects. Validated as biomarker of mitochondrial myopathy but low diagnostic specificity | Forsstrom et al. [42] |
| | GDF15 | Tested in different PMDs, particularly TK2 deficiency | Elevated in PMDs but low specificity; correlates with disease severity and decreases with therapy in TK2 deficiency | Tsygnkova et al. [52], Bermejo-Guerrero et al. [53] |
| | Gelsolin | Tested in a cohort of different PMDs, including CPEO, MELAS | Reduced. Evaluated in association with GDF15, can improve diagnostic accuracy | Penas et al. [56] |
| | NADH-related reductive stress markers and $NAD^+$ | Tested in a cohort of different PMDs, particularly with mitochondrial myopathy | Circulating signatures correlate with disease burden; Reduced $NAD^+$, explored as biomarker and target in niacin trial | Pirinen et al., [66] |
| | Neurofilament light chain (NfL)* | Tested in different disorders, particularly in MELAS | Serum NfL elevated in neuroaxonal injury; biomarker potential. In CSF correlated with disease severity | Varhaug et al. [74]; Sofou et al. [73] |
| | Phosphorylated neurofilament heavy chain (pNfH) | LHON | Suggested as marker of axonal degeneration after visual loss function | Guy et al. [75] |
| | Interferon-stimulated genes | Tested in a cohort of pediatric PMDs | Upregulated; may reflect immune dysregulation | Keshavan et al. [81] |
| | Cytokines | PMDs | May reflect inflammatory activation, cytokine alterations. Need further validation | Primiano et al. [83] |
| | Chitotriosidase (CHIT1) | PMDs | Elevated; candidate biomarker for PMDs | Foerster et al. [80] |
| | Arachidonic acid metabolism, bile acid biosynthesis | MNGIE | Significant upregulation; disease-specific metabolic pattern, potentially specific for MNGIE | Bax et al. [64] |
| | miRNA | MNGIE | miR-192-5p, miR-193a-5p, miR-194-5p, miR-215-5p and miR-34a-5p in plasma; miR-192-5p, miR-194-5p, miR-34a-5p: potentially specific for MNGIE | Mencias et al. [84]; |
| | | PMD patients with sensorineural hearing loss | miR-34a, miR-29b | Marozzo et al. [86] |

**Table 2** (continued)

| Organ | Analyte/Biomarker | Mitochondrial condition(s) | Findings and applications/limits | References |
|---|---|---|---|---|
| | Circulating cell-free mitochondrial DNA (ccf-mtDNA)* | MELAS, MERRF | Elevated during acute episodes; indicates mitochondrial stress | Maresca et al. [70] |
| Saliva | GDF15 | Tested in different PMDs | Detectable and dynamic; correlates with stress, utility might stand in its minimally invasiveness | Huang et al. [55] |

Organic acids and acylcarnitines (which could be useful in the differential diagnosis with other metabolic conditions) are not mentioned in the table

*CK* Creatine kinase, *CPEO* Chronic Progressive External Ophthalmoplegia, *FGF21* Fibroblast Growth Factor 21, *GDF15* Growth Differentiation Factor 15, *LHON* Leber Hereditary Optic Neuropathy, *MELAS* Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes, *MERRF* Myoclonic Epilepsy with Ragged-Red Fibers, *MNGIE* Mitochondrial Neurogastrointestinal Encephalopathy, *TK2* Thymidine Kinase 2

*Tested also in Cerebrospinal fluid (CSF)

measure of skeletal muscle mitochondrial oxidative capacity and has been explored as pharmacodynamic biomarker in preliminary interventional studies [108]. Despite this potential, broader clinical implementation remains limited by the need for specialized hardware, standardized protocols, and expertise in spectral analysis [109, 110] and it is not widely used in longitudinal studies.

Diffusion MRI and arterial spin labeling (ASL) contribute complementary information about microstructure, cytotoxic stress and perfusion, particularly relevant for stroke-like lesions in MELAS, but insufficiently specific for PMDs [111]. Diffusion-weighted MRI (DWI) reveals characteristics abnormalities in MELAS, reflecting metabolic edema rather than vascular ischemia [112]. In Leigh syndrome and Kearns–Sayre syndrome, DWI hyperintensities with abnormal ADC values have also been noted in the brainstem [113].

Molecular PET imaging approaches enhance biomarker sensitivity and specificity, with a potential for directly measuring disease-relevant aspects of mitochondrial biology: brain translocator protein (TSPO) PET scans are typically used to measure neuro-inflammation and microglial activation in a wide range of neurologic conditions [114, 115]. Interestingly, TSPO is an outer mitochondrial membrane protein, and could therefore be affected by impaired mitochondrial function or mass. TSPO PET with the $^{11}$C-PK11195 ligand appears to show signal changes that correlate with disease severity, and may detect abnormality in absence of MRI changes [116]. Multiple case reports on patients with MELAS indicate that FDG-PET reveals regional glucose hypo- or hyper-metabolism [117, 118].

Challenges persist, in particular for PET, with substantial costs associated with tracer production and imaging. Genetic variants can influence tracer binding [119], there are partial volume effects [120], and mitochondrial dysfunction can vary across tissue types, which together complicate signal interpretation and routine clinical application. Nevertheless, as highlighted in recent reviews [87, 103, 121], many of these imaging approaches may ultimately support earlier diagnosis, stratify disease subtypes, or track treatment response. A summary of the main imaging techniques used in patients with PMDs is presented in Table 3.

## Emerging non-invasive biomarkers and imaging approaches with promise in PMDs

Beyond traditional serum and neuroimaging markers, several innovative methodologies are emerging with the potential to provide sensitive, real-time readouts of mitochondrial dysfunction, metabolic capacity, and tissue-level bioenergetics. These approaches may offer a viable path toward objective

**Table 3** List of the main central nervous system imaging techniques used in patients with Primary Mitochondrial Diseases (PMDs)

| Organ | Technique | Sequence | Mitochondrial condition | Observation | References |
|---|---|---|---|---|---|
| Brain | MRI | T2/FLAIR | Wide range of PMDs | Symmetric hyper-intensities in white and deep gray matter; Cerebellar atrophy, for example in POLG mutations; Prominent leukoencephalopathy, for example in MNGIE; Symmetric hyper-intensity of basal ganglia, for example in Leigh Syndrome; Focal gray matter lesions, for example in stroke-like episodes caused by MELAS, typically in temporal, parietal, and occipital lobes; Developmental abnormalities, for example in Pyruvate Dehydrogenase deficiency, etc | Extensively reviewed in Saneto et al. [88] |
| | | DWI | MELAS (stroke-like episodes) | Stroke-like lesions typically demonstrate normal or increased apparent diffusion coefficient (ADC) values, indicative of vasogenic edema, rather than restricted diffusion in ischemic stroke | Oppenheim et al. [112] |
| | | Arterial Spin Labeling (ASL) | MELAS (stroke-like episodes) | Peripheral hyper-perfusion observed in stroke-like lesions assists in differentiating from acute ischemic stroke | Li et al. [111] |
| | MRS | $^{1}$H-MRS | MELAS | An elevated lactate peak and reduced N-acetyl-aspartate (NAA) constitute a metabolic profile indicative of mitochondrial dysfunction | Abe et al. [100] |
| | | | Kearns–Sayre syndrome/Pearson syndrome | An increase in lactate within the affected white matter | Kapeller et al. [99] |
| | | | Leigh syndrome | Elevated lactate is detected in both affected gray and white matter, often accompanied by increased choline levels | Sijens et al. [98] |
| | | $^{31}$P-MRS | Mitochondrial myopathies | Prolonged post-exercise Phosphocreatine (PCr) recovery | Jeppesen et al. [107] |
| | PET | TSPO PET | Tested in a cohort of different PMDs | Regional alterations in TSPO ([$^{11}$C] PK11195) binding may correspond to phenotypes or clinical severity | van den Ameele et al. [116] |
| | | FDG-PET | MELAS (stroke-like episodes) | Regional hypo-metabolism or hyper-metabolism, dependent on disease stage or presence of seizures | Liu et al. [117] |
| Eye | MRI | | CPEO | Marked atrophy of the extraocular muscles | Yu-Wai-Man et al. [89] |

*CPEO* Chronic Progressive External Ophthalmoplegia, *DWI* Diffusion-Weighted Imaging, *FDG* Fluorodeoxyglucose, *FLAIR* Fluid-Attenuated Inversion Recovery, *MELAS* Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes, *MRI* Magnetic Resonance Imaging, *MRS* Magnetic Resonance Spectroscopy, *PET* Positron Emission Tomography, *POLG* DNA Polymerase, Gamma, TS*P*O Translocator protein

stratification and functional endpoints for upcoming clinical trials, although many are still constrained to preclinical models or other diseases and have not been tested in patients with PMDs.

Among laboratory biomarkers, circulating axonal cytoskeletal proteins such as peripherin have emerged as promising biomarkers of peripheral nervous damage [122, 123], paving the way for PMDs presenting with peripheral neuropathy. Ongoing studies, for example in asymptomatic mtDNA variant carriers for LHON, aim to identify signatures predictive of phenotypic conversion [124].

A wide range of imaging techniques is also emerging, with promise as novel biomarkers in PMDs. Near-Infrared Spectroscopy (NIRS) with vascular occlusion testing provides a non-invasive method to assess muscle oxygenation, hemodynamics, and oxidative metabolism. In pediatric

populations with PMDs and neuro-genetic disorders, NIRS-derived parameters have shown sensitivity to impaired OXPHOS in small clinical cohorts of patients with mitochondrial or other neuromuscular disorders, correlating with clinical severity and functional limitations [125]. The portability and tolerability of NIRS could make it attractive for longitudinal clinical monitoring, including in pediatric populations [126].

Other imaging techniques seek to enhance spatial specificity and provide mechanistic insights into metabolic alterations. Chemical Exchange Saturation Transfer (CEST), has demonstrated potential in an Ndufs4 knockout mouse model for mapping intracellular lactate with higher resolution than MRS [127], but has not yet been systematically evaluated in patients. Deuterium Metabolic Imaging (DMI) is an MRI-based technology that provides a dynamic map of glucose utilization and downstream metabolites such as glutamate/glutamine or lactate by tracing deuterated glucose, offering insight into real-time tissue bioenergetics. Though only recently applied in neurological disorders, such as Alzheimer's disease and glioblastoma [128, 129], DMI holds promise for PMDs by enabling direct assessment of glycolytic flux and OXPHOS that could serve as quantitative biomarkers in trials targeting cerebral energy metabolism [129, 130]. However, studies in PMDs are currently lacking. Another emerging MRI-based technique is Muscle Oxygen Extraction Fraction (OEF), which measures the efficiency of oxygen utilization in muscle and might detect changes in early disease stages [132, 133] although these tracers are currently being evaluated primarily in research settings and have not yet been systematically applied in PMD cohorts. Mitochondrial-targeted tracers for PET imaging, such as $^{18}$F-BCPP-EF, are in development to enable in vivo quantification of complex I activity [131].

Multi-Spectral Optoacoustic Tomography (MSOT) enables high-resolution real-time visualization of tissue structure and function based on photoacoustic contrast. MSOT allows non-invasive interrogation of physiological parameters linked to mitochondrial function, such as tissue oxygen extraction and microvascular dynamics. Although experience in PMDs remains limited, studies in related neuromuscular diseases as Duchenne Muscular Dystrophy [115], Spinal Muscular Atrophy [134] or Pompe Disease [135], support its potential as a future PMD monitoring or response biomarker. Preclinical studies have demonstrated its feasibility in mouse models for quantification of optoacoustic signatures from metabolically active organs with excellent temporal and spatial resolution [136, 137], ultimately appearing a potential bedside-compatible functional imaging biomarker for tissue-level bioenergetics [138], as demonstrated in a recent exploratory study in patients with m.3243 A>G pathogenic variants [REF].

Finally, Raman spectroscopy of tissue samples or in vivo with fiber-optics may capture protein conformation, metabolic signatures, and structural alterations that precede or accompany muscle pathology. Fiber-optic Raman spectroscopy was shown to differentiate between healthy and diseased muscle in mouse, offering a minimally invasive alternative to standard histopathology [139], and might even be combined with muscle electrophysiology [140].

Overall, while these emerging imaging approaches provide exciting opportunities to interrogate mitochondrial biology in vivo, most remain at an early stage of development. Systematic validation in well-characterized PMD cohorts, standardization of acquisition protocols, and demonstration of reproducibility across centers will be essential before these techniques can be considered robust biomarkers for clinical settings and trials.

## Conclusion

In clinical practice, the management of PMDs often remains challenging, in particular due to marked phenotypic and genetic heterogeneity and fluctuating disease trajectories. To date, no single biomarker has proven sufficiently sensitive, specific, and broadly applicable to meet all the diverse diagnostic and longitudinal needs of these heterogeneous disorders. Traditional imaging, biochemical and functional measures remain essential in routine clinical care, yet their limitations highlight the need for complementary approaches. Recent advances in non-invasive laboratory biomarkers, digital and wearable technologies, and metabolic and molecular imaging, although still requiring validation in many cases, provide clinicians with an expanding toolbox capable of capturing complementary dimensions of mitochondrial dysfunction, tissue injury, and functional impairment. At this stage, rather than replacing traditional measures, these biomarkers should be viewed as adjunct options that can refine diagnostic confidence and support clinical monitoring. They are likely to play an increasingly important role in patient stratification and in providing objective readouts of disease activity and treatment response, particularly in clinical trials.

Looking ahead, validation of partially established biomarkers remains a priority, such as NfL in serum, brain, or muscle MRS imaging, or PET tracers. These should be validated in larger cohorts, together with harmonization of image acquisition protocols, and improved reproducibility of digital health technology endpoints. In parallel, exploratory technologies, including Raman spectroscopy, MSOT, DMI, and novel PET tracers should be progressively translated from preclinical studies into clinical evaluation. Over the medium term, prospective longitudinal

studies will be essential to determine biomarker responsiveness, predictive value, and utility as pharmacodynamic endpoints in clinical trials. This should occur alongside efforts to develop disorder- and treatment-specific biomarker profiles, leveraging deep phenotyping and machine learning to inform patient stratification and therapeutic decision-making.

Finally, long-term progress will likely rely on integration of both generic and disorder-specific biomarkers into compound measures, for which large-scale cohorts, deep phenotyping and machine learning approaches may prove useful. Given the distinct pathophysiology and therapeutic strategies of individual syndromic groups, the pursuit of universal biomarkers across all PMDs is likely to remain challenging. Biomarker development should therefore align closely with disease pathogenesis and the key metabolic and biochemical pathways amenable to therapy development, or aim to measure clinical phenotypes with direct relevance for patients and their quality of life [15]. As targeted treatments progress toward clinical application, establishing and harmonizing such multidimensional, disorder- and treatment-specific biomarker profiles will be essential to enable rigorous clinical trials and to advance clinical patient care.

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**Data availability** Not applicable.

## Declarations

**Conflicts of interest** The authors declare no competing interests.

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## References

1. Gorman GS, Schaefer AM, Ng Y et al (2015) Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Ann Neurol 77:753–759. https://doi.org/10.1002/ana.24362
2. Ng YS, Gorman GS, Mcfarland R et al (2021) Mitochondrial disease in adults: recent advances and future promise. Lancet Neurol 20(7):573–84
3. Schon KR, Ratnaike T, van den Ameele J et al (2020) Mitochondrial diseases: a diagnostic revolution. Trends Genet 36:702–717
4. Suomalainen A, Battersby BJ (2018) Mitochondrial diseases: the contribution of organelle stress responses to pathology. Nat Rev Mol Cell Biol 19:77–92
5. Stewart JB, Chinnery PF (2021) Extreme heterogeneity of human mitochondrial DNA from organelles to populations. Nat Rev Genet 22:106–118
6. Ball M, van Bergen NJ, Compton AG et al (2025) Therapies for mitochondrial disease: past, present, and future. J Inherit Metab Dis 48:e70065
7. Yu-Wai-Man P, Carelli V, Newman NJ et al (2024) Therapeutic benefit of idebenone in patients with Leber hereditary optic neuropathy: the LEROS nonrandomized controlled trial. Cell Rep Med. https://doi.org/10.1016/j.xcrm.2024.101437
8. Thompson WR, Manuel R, Abbruscato A et al (2024) Long-term efficacy and safety of elamipretide in patients with Barth syndrome: 168-week open-label extension results of TAZPOWER. Genet Med. https://doi.org/10.1016/j.gim.2024.101138
9. Domínguez-González C, Chiang C, Colson AO et al (2025) Pyrimidine nucleos(t)ide therapy in patients with thymidine kinase 2 deficiency a multicenter retrospective chart review study. Neurology. https://doi.org/10.1212/WNL.0000000000213908
10. U.S. Food and Drug Administration. FDA approves 1st drug for thymidine kinase 2 deficiency, a very raremitochondrial disease [Internet]. Silver Spring (MD): FDA; 2025 Nov 3 [cited 2026 Apr 7]. Available from: https://www.fda.gov/drugs/news-events-human-drugs/fda-approves-1st-drug-thymidine-kinase-2-deficiency-very-rare-mitochondrial-disease
11. Karaa A, Klopstock T (2023) Clinical trials in mitochondrial diseases. Handbook of clinical neurology. Elsevier B.V, Amsterdam, pp 229–250
12. Newman NJ, Biousse V, Yu-Wai-Man P et al (2025) Meta-analysis of treatment outcomes for patients with m.11778G>A MT-ND4 Leber hereditary optic neuropathy. Surv Ophthalmol 70:283–295
13. Leslie M (2025) First approved drug for mitochondrial disease could pave way for more treatments. Science (1979) 390:114–115
14. Mancuso M, Lopriore P, Semmler L, Kornblum C (2025) 280th ENMC International Workshop: The ERN EURO-NMD mitochondrial diseases working group; diagnostic criteria and outcome measures in primary mitochondrial myopathies.

Hoofddorp, the Netherlands, 22–24 November 2024. Neuromuscul Disord 50:105340
15. Thomas RH, Hunter A, Butterworth L et al (2022) Research priorities for mitochondrial disorders: current landscape and patient and professional views. J Inherit Metab Dis 45:796–803. https://doi.org/10.1002/jimd.12521
16. Atkinson AJ, Colburn WA, DeGruttola VG et al (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69:89–95
17. Steele HE, Horvath R, Lyon JJ, Chinnery PF (2017) Monitoring clinical progression with mitochondrial disease biomarkers. Brain 140:2530–2540. https://doi.org/10.1093/brain/awx168
18. Hubens WHG, Vallbona-Garcia A, de Coo IFM et al (2022) Blood biomarkers for assessment of mitochondrial dysfunction: an expert review. Mitochondrion 62:187–204
19. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. SilverSpring (MD): Food and Drug Administration (US); 2016. Co-published by National Institutes of Health (US), Bethesda(MD). Available from: https://www.ncbi.nlm.nih.gov/books/NBK326791/toc/
20. Pizzamiglio C, Vernon HJ, Hanna MG, Pitceathly RDS (2022) Designing clinical trials for rare diseases: unique challenges and opportunities. Nat Rev Methods Primers 2:13
21. Karaa A, Johnson N, Clarkson I et al (2023) Characterization of fatigue in primary mitochondrial myopathies: findings from a qualitative interview study. Neurol Clin Pract. https://doi.org/10.1212/CPJ.0000000000200229
22. Flickinger J, Fan J, Wellik A et al (2021) Development of a mitochondrial myopathy-composite assessment tool. JCSM Clin Rep 6(4):109–27
23. Jeppesen TD, Madsen KL, Poulsen NS et al (2021) Exercise testing, physical training and fatigue in patients with mitochondrial myopathy related to mtDNA mutations. J Clin Med. https://doi.org/10.3390/jcm10081796
24. Barroso de Queiroz Davoli G, Bartels B, Mattiello-Sverzut AC, Takken T (2021) Cardiopulmonary exercise testing in neuromuscular disease: a systematic review. Expert Rev Cardiovasc Ther 19:975–991
25. Montano V, Lopriore P, Gruosso F et al (2022) Primary mitochondrial myopathy: 12-month follow-up results of an Italian cohort. J Neurol 269:6555–6565. https://doi.org/10.1007/s00415-022-11324-3
26. Løkken N, Khawajazada T, Storgaard JH et al (2021) No effect of resveratrol in patients with mitochondrial myopathy: a cross-over randomized controlled trial. J Inherit Metab Dis 44:1186–1198. https://doi.org/10.1002/jimd.12393
27. Bergs PMJ, Maas DM, Janssen MCH, Groothuis JT (2022) Feasible and clinical relevant outcome measures for adults with mitochondrial disease. Mol Genet Metab 135:102–108. https://doi.org/10.1016/j.ymgme.2021.12.010
28. Bortolani S, Brusa C, Rolle E et al (2022) Technology outcome measures in neuromuscular disorders: a systematic review. Eur J Neurol 29:1266–1278
29. Servais L, Yen K, Guridi M et al (2022) Stride velocity 95th centile: insights into gaining regulatory qualification of the first wearable-derived digital endpoint for use in Duchenne Muscular Dystrophy trials. J Neuromuscul Dis 9:335–346
30. Apabhai S, Gorman GS, Sutton L et al (2011) Habitual physical activity in mitochondrial disease. PLoS ONE. https://doi.org/10.1371/journal.pone.0022294
31. Galna B, Newman J, Jakovljevic DG et al (2014) Discrete gait characteristics are associated with m.3243A>G and m.8344A>G variants of mitochondrial disease and its pathological consequences. J Neurol 261:73–82. https://doi.org/10.1007/s00415-013-7129-2
32. Koene S, Stolwijk NM, Ramakers R et al (2018) Quantification of gait in children with mitochondrial disease. J Inherit Metab Dis 41:731–740. https://doi.org/10.1007/s10545-018-0148-5
33. Ramakers R, Koene S, Groothuis JT et al (2017) Quantification of gait in mitochondrial m.3243A > G patients: a validation study. Orphanet J Rare Dis. https://doi.org/10.1186/s13023-017-0644-y
34. Steele H, Gomez-Duran A, Pyle A et al (2020) Metabolic effects of bezafibrate in mitochondrial disease. EMBO Mol Med. https://doi.org/10.15252/emmm.201911589
35. Haas RH, Parikh S, Falk MJ et al (2008) The in-depth evaluation of suspected mitochondrial disease. Mol Genet Metab 94:16–37. https://doi.org/10.1016/j.ymgme.2007.11.018
36. Debray FG, Mitchell GA, Allard P et al (2007) Diagnostic accuracy of blood lactate-to-pyruvate molar ratio in the differential diagnosis of congenital lactic acidosis. Clin Chem 53:916–921. https://doi.org/10.1373/clinchem.2006.081166
37. Parikh S, Goldstein A, Koenig MK et al (2015) Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med 17:689–701
38. Shayota BJ (2024) Biomarkers of mitochondrial disorders. Neurotherapeutics 21:e00325
39. Paredes-Fuentes AJ, Oliva C, Urreizti R et al (2023) Laboratory testing for mitochondrial diseases: biomarkers for diagnosis and follow-up. Crit Rev Clin Lab Sci 60:270–289
40. Gervasoni J, Primiano A, Cicchinelli M et al (2024) Mitochondrial biomarkers in the omics era: a clinical-pathophysiological perspective. Int J Mol Sci. https://doi.org/10.3390/ijms25094855
41. Tyynismaa H, Carroll CJ, Raimundo N et al (2010) Mitochondrial myopathy induces a starvation-like response. Hum Mol Genet 19:3948–3958. https://doi.org/10.1093/hmg/ddq310
42. Forsström S, Jackson CB, Carroll CJ et al (2019) Fibroblast Growth Factor 21 drives dynamics of local and systemic stress responses in mitochondrial myopathy with mtDNA deletions. Cell Metab 30:1040-1054.e7. https://doi.org/10.1016/j.cmet.2019.08.019
43. Lehtonen JM, Forsström S, Bottani E et al (2016) FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders. Neurology 87:2290–2299. https://doi.org/10.1212/WNL.0000000000003374
44. Buzkova J, Nikkanen J, Ahola S et al (2018) Metabolomes of mitochondrial diseases and inclusion body myositis patients: treatment targets and biomarkers. EMBO Mol Med. https://doi.org/10.15252/emmm.201809091
45. Sevastianova K, Yki-Järvinen H, Smeitink J, et al (2011) FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain defi ciencies: a diagnostic study. www.thelancet.com/neurology https://doi.org/10.1016/S1474
46. Khan NA, Nikkanen J, Yatsuga S et al (2017) mTORC1 regulates mitochondrial integrated stress response and mitochondrial myopathy progression. Cell Metab 26:419-428.e5. https://doi.org/10.1016/j.cmet.2017.07.007
47. Riley LG, Nafisinia M, Menezes MJ et al (2022) FGF21 outperforms GDF15 as a diagnostic biomarker of mitochondrial disease in children. Mol Genet Metab 135:63–71. https://doi.org/10.1016/j.ymgme.2021.12.001
48. Tekin Neijmann S, Gunes D, Karaca M et al (2025) Evaluation of serum FGF21 levels in patients with mitochondrial aminoacyl-tRNA synthetase deficiency. Int J Mol Sci. https://doi.org/10.3390/ijms26199525
49. Kalko SG, Paco S, Jou C et al (2014) Transcriptomic profiling of TK2 deficient human skeletal muscle suggests a role for the p53 signalling pathway and identifies growth and differentiation factor-15 as a potential novel biomarker for mitochondrial myopathies. BMC Genom. https://doi.org/10.1186/1471-2164-15-91

50. Steele HE, Horvath R, Taylor RW (2016) The swinging pendulum of biomarkers in mitochondrial disease. Neurology 87:2286–2287
51. Yatsuga S, Fujita Y, Ishii A et al (2015) Growth differentiation factor 15 as a useful biomarker for mitochondrial disorders. Ann Neurol 78:814–823. https://doi.org/10.1002/ana.24506
52. Tsygankova PG, Itkis YS, Krylova TD et al (2019) Plasma FGF-21 and GDF-15 are elevated in different inherited metabolic diseases and are not diagnostic for mitochondrial disorders. J Inherit Metab Dis 42:918–933. https://doi.org/10.1002/jimd.12142
53. Bermejo-Guerrero L, Hernández-Voth A, Serrano-Lorenzo P et al (2024) Remarkable clinical improvement with oral nucleoside treatment in a patient with adult-onset TK2 deficiency: a case report. Mitochondrion. https://doi.org/10.1016/j.mito.2024.101879
54. Martín-Jimenez P, Bermejo-Guerrero L, Navarro-Riquelme M et al (2025) Comprehensive analysis of GDF15 as a biomarker in primary mitochondrial myopathies. Mol Genet Metab. https://doi.org/10.1016/j.ymgme.2025.109023
55. Huang Q, Trumpff C, Monzel AS et al (2025) The mitochondrial disease biomarker GDF15 is dynamic, quantifiable in saliva, and correlates with disease severity. Mol Genet Metab. https://doi.org/10.1016/j.ymgme.2025.109179
56. Peñas A, Fernández-De la Torre M, Laine-Menéndez S et al (2021) Plasma gelsolin reinforces the diagnostic value of FGF-21 and GDF-15 for mitochondrial disorders. Int J Mol Sci. https://doi.org/10.3390/ijms22126396
57. Correia SP, Moedas MF, Taylor LS et al (2024) Quantitative proteomics of patient fibroblasts reveal biomarkers and diagnostic signatures of mitochondrial disease. JCI Insight. https://doi.org/10.1172/jci.insight.178645
58. Li Y, Wang D, Zhou M et al (2025) Hypocitrullinemia as an early diagnostic biomarker for MT-ATP6 mitochondrial diseases. J Mol Neurosci. https://doi.org/10.1007/s12031-025-02440-6
59. Carli S, Levarlet A, Diodato D et al (2025) Natural history of patients with mitochondrial ATPase deficiency due to pathogenic variants of MT-ATP6 and MT-ATP8. Neurology. https://doi.org/10.1212/WNL.0000000000213462
60. Manzoni E, Carli S, Gaignard P et al (2024) Deoxyguanosine kinase deficiency: natural history and liver transplant outcome. Brain Commun. https://doi.org/10.1093/braincomms/fcae160
61. Hall AM, Vilasi A, Garcia-Perez I et al (2015) The urinary proteome and metabonome differ from normal in adults with mitochondrial disease. Kidney Int 87:610–622. https://doi.org/10.1038/ki.2014.297
62. Cicchinelli M, Primiano G, Canu F et al (2025) Urinary multiomics profiling reveals systemic molecular alterations in Progressive External Ophthalmoplegia. Int J Mol Sci 26:11257. https://doi.org/10.3390/ijms262311257
63. Cao B, Lu H, Liu P et al (2025) Serum metabolomics signature of maternally inherited diabetes and deafness by gas chromatography–time of flight mass spectrometry. J Diabetes Investig 16:146–153. https://doi.org/10.1111/jdi.14334
64. Bax BE, Uçar SK (2025) Untargeted plasma metabolomics extends the biomarker profile of mitochondrial neurogastrointestinal encephalomyopathy. Int J Mol Sci. https://doi.org/10.3390/ijms26189107
65. Sharma R, Reinstadler B, Engelstad K et al (2021) Circulating markers of NADH-reductive stress correlate with mitochondrial disease severity. J Clin Invest. https://doi.org/10.1172/JCI136055
66. Pirinen E, Auranen M, Khan NA et al (2020) Niacin cures systemic NAD+ deficiency and improves muscle performance in Adult-Onset Mitochondrial Myopathy. Cell Metab 31:1078-1090.e5. https://doi.org/10.1016/j.cmet.2020.04.008
67. Hirano M, Carelli V, De Giorgio R et al (2021) Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): position paper on diagnosis, prognosis, and treatment by the MNGIE International Network. J Inherit Metab Dis 44:376–387
68. De Giorgio R, Pironi L, Rinaldi R et al (2016) Liver transplantation for mitochondrial neurogastrointestinal encephalomyopathy. Ann Neurol 80:448–455. https://doi.org/10.1002/ana.24724
69. Halter JP, Michael W, Schüpbach M et al (2015) Allogeneic haematopoietic stem cell transplantation for mitochondrial neurogastrointestinal encephalomyopathy. Brain 138:2847–2858. https://doi.org/10.1093/brain/awv226
70. Bax BE, Bain MD, Scarpelli M et al (2013) Clinical and biochemical improvements in a patient with MNGIE following enzyme replacement. Neurology 81:1269–1271. https://doi.org/10.1212/WNL.0b013e3182a6cb4b
71. Torres-Torronteras J, Viscomi C, Cabrera-Pérez R et al (2014) Gene therapy using a liver-targeted AAV vector restores nucleoside and nucleotide homeostasis in a murine model of MNGIE. Mol Ther 22:901–907. https://doi.org/10.1038/mt.2014.6
72. Brevini T, Swift L, Reynolds H et al (2025) Successful AAV8 gene therapy on hepatic ex situ machine perfusion for mitochondrial neurogastrointestinal encephalomyopathy. J Hepatol 83:1218–1225. https://doi.org/10.1016/j.jhep.2025.07.022
73. Sofou K, Shahim P, Tulinius M et al (2019) Cerebrospinal fluid neurofilament light is associated with survival in mitochondrial disease patients. Mitochondrion 46:228–235. https://doi.org/10.1016/j.mito.2018.07.002
74. Varhaug KN, Hikmat O, Nakkestad HL et al (2021) Serum biomarkers in primary mitochondrial disorders. Brain Commun. https://doi.org/10.1093/braincomms/fcaa222
75. Guy J, Shaw G, Ross-Cisneros FN et al (2008) Phosphorylated neurofilament heavy chain is a marker of neurodegeneration in Leber hereditary optic neuropathy (LHON). Mol Vis 14:2443
76. Zheng YS, Sun C, Wang R et al (2021) Neurofilament light is a novel biomarker for mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes. Sci Rep. https://doi.org/10.1038/s41598-021-81721-7
77. Bermejo-Guerrero L, Restrepo-Vera JL, Martin-Jimenez P et al (2026) Clinical heterogeneity and candidate biomarkers in POLG-related mitochondrial disease. Neurol Genet. https://doi.org/10.1212/NXG.0000000000200365
78. Maresca A, Del Dotto V, Romagnoli M et al (2020) Expanding and validating the biomarkers for mitochondrial diseases. J Mol Med 98:1467–1478. https://doi.org/10.1007/s00109-020-01967-y
79. Trifunov S, Paredes-Fuentes AJ, Badosa C et al (2021) Circulating cell-free mitochondrial DNA in cerebrospinal fluid as a biomarker for mitochondrial diseases. Clin Chem 67:1113–1121. https://doi.org/10.1093/clinchem/hvab091
80. Foerster L, Scholle L, Mayer T et al (2025) Serum chitotriosidase-1 (CHIT1) as candidate biomarker for mitochondriopathies. J Neurol. https://doi.org/10.1007/s00415-025-12916-5
81. Keshavan N, Mhaldien L, Gilmour K, Rahman S (2024) Interferon stimulated gene expression is a biomarker for primary mitochondrial disease. Ann Neurol 96:1185–1200. https://doi.org/10.1002/ana.27081
82. Warren EB, Gordon-Lipkin EM, Cheung F et al (2023) Inflammatory and interferon gene expression signatures in patients with mitochondrial disease. J Transl Med. https://doi.org/10.1186/s12967-023-04180-w
83. Primiano G, Plantone D, Piro G et al (2023) Inflammatory profile in mitochondrial diseases: A cohort study. Eur J Neurol 30:3409–3410
84. Mencias M, Levene M, Blighe K, Bax BE (2021) Circulating mirnas as biomarkers for mitochondrial neuro-gastrointestinal encephalomyopathy. Int J Mol Sci. https://doi.org/10.3390/ijms22073681

85. Meseguer S, Martínez-Zamora A, García-Arumí E, Andreu AL, Armengod ME (2015) The ROS-sensitive microRNA-9/9*controls the expression of mitochondrial tRNA-modifying enzymes and is involved in the molecular mechanism ofMELAS syndrome. Hum Mol Genet 24(1):167-184. https://doi.org/10.1093/hmg/ddu427
86. Marozzo R, Pegoraro V, Dipietro L et al (2020) Can miR-34a be suitable for monitoring sensorineural hearing loss in patients with mitochondrial disease? A case series. Int J Neurosci 130:1272–1277. https://doi.org/10.1080/00207454.2020.1731505
87. Distelmaier F, Klopstock T (2023) Neuroimaging in mitochondrial disease. Handbook of clinical neurology. Elsevier B.V, Amsterdam, pp 173–185
88. Saneto RP, Friedman SD, Shaw DWW (2008) Neuroimaging of mitochondrial disease. Mitochondrion 8:396–413
89. Yu-Wai-Man C, Smith FE, Firbank MJ et al (2013) Extraocular muscle atrophy and central nervous system involvement in chronic progressive external ophthalmoplegia. PLoS One. https://doi.org/10.1371/journal.pone.0075048
90. Ardissone A, Bruno C, Diodato D et al (2021) Clinical, imaging, biochemical and molecular features in Leigh syndrome: a study from the Italian network of mitochondrial diseases. Orphanet J Rare Dis. https://doi.org/10.1186/s13023-021-02029-3
91. Manuel Barragán-Campos H, Vallée J-N, Lô D et al (2005) Brain magnetic resonance imaging findings in patients with mitochondrial cytopathies. Arch Neurol 62(5):737–42
92. Lerman-Sagie T, Leshinsky-Silver E, Watemberg N et al (2005) White matter involvement in mitochondrial diseases. Mol Genet Metab 84:127–136
93. Tzoulis C, Engelsen BA, Telstad W (2006) The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases. Brain 129:1685–1692. https://doi.org/10.1093/brain/awl097
94. Farina L, Chiapparini L, Uziel G et al (2002) MR Findings in Leigh Syndrome with COX Deficiency and SURF-1 Mutations. Am J Neuroradiol 23(7):1095–100
95. Florian A, Ludwig A, Stubbe-Dräger B et al (2015) Characteristic cardiac phenotypes are detected by cardiovascular magnetic resonance in patients with different clinical phenotypes and genotypes of mitochondrial myopathy. J Cardiovasc Magn Reson. https://doi.org/10.1186/s12968-015-0145-x
96. Gray R, Kantartzı V, Lopes LR et al (2025) Cardiac magnetic resonance findings in mitochondrial disease: a guide for clinicians. Eur Heart J Imaging Methods Pract. https://doi.org/10.1093/ehjimp/qyaf134
97. Gramegna LL, Evangelisti S, Di Vito L et al (2021) Brain MRS correlates with mitochondrial dysfunction biomarkers in MELAS-associated mtDNA mutations. Ann Clin Transl Neurol 8:1200–1211. https://doi.org/10.1002/acn3.51329
98. Sijens PE, Smit GPA, Rödiger LA et al (2008) MR spectroscopy of the brain in Leigh syndrome. Brain Dev 30:579–583. https://doi.org/10.1016/j.braindev.2008.01.011
99. Kapeller P, Fazekas F, Offenbacher H et al (1996) Magnetic resonance imaging and spectroscopy of progressive cerebral involvement in Keams Sayre Syndrome. J Neurol Sci 135(2):126–30
100. Abe K, Yoshimura H, Tanaka H et al (2004) Comparison of conventional and diffusion-weighted MRI and proton MR spectroscopy in patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like events. Neuroradiology 46:113–117. https://doi.org/10.1007/s00234-003-1138-2
101. Lunsing RJ, Strating K, de Koning TJ, Sijens PE (2017) Diagnostic value of MRS-quantified brain tissue lactate level in identifying children with mitochondrial disorders. Eur Radiol 27:976–984. https://doi.org/10.1007/s00330-016-4454-8
102. Chi CS, Lee HF, Tsai CR et al (2011) Lactate peak on brain MRS in children with syndromic mitochondrial diseases. J Chin Med Assoc 74:305–309. https://doi.org/10.1016/j.jcma.2011.05.006
103. Kielty K, Collyer J, Ganesh KD et al (2025) Spectrum of clinical neuroimaging in mitochondrial disorders: a neuroanatomical approach. Pediatr Radiol 55:1350–1365
104. Lin A, Andronesi O, Bogner W et al (2021) Minimum reporting standards for in vivo magnetic resonance spectroscopy (MRSinMRS): experts' consensus recommendations. NMR Biomed. https://doi.org/10.1002/nbm.4484
105. Lodi R, Rajagopalan B, Blamire AM et al (2004) Abnormal cardiac energetics in patients carrying the A3243G mtDNA mutation measured in vivo using phosphorus MR spectroscopy. Biochim Biophys Acta Bioenerg 1657:146–150. https://doi.org/10.1016/j.bbabio.2004.05.003
106. Kemp GJ, Meyerspeer M, Moser E (2007) Absolute quantification of phosphorus metabolite concentrations in human muscle in vivo by 31P MRS: a quantitative review. NMR Biomed 20:555–565
107. Jeppesen TD, Quistorff B, Wibrand F, Vissing J (2007) 31P-MRS of skeletal muscle is not a sensitive diagnostic test for mitochondrial myopathy. J Neurol 254:29–37. https://doi.org/10.1007/s00415-006-0229-5
108. Roshanravan B, Liu SZ, Ali AS et al (2021) In vivo mitochondrial ATP production is improved in older adult skeletal muscle after a single dose of elamipretide in a randomized trial. PLoS One. https://doi.org/10.1371/journal.pone.0253849
109. Kumar V, Chang H, Reiter DA et al (2017) Phosphorus-31 magnetic resonance spectroscopy: A tool for measuring in vivo mitochondrial oxidative phosphorylation capacity in human skeletal muscle. J Vis Exp. https://doi.org/10.3791/54977
110. Meyerspeer M, Boesch C, Cameron D et al (2021) 31P magnetic resonance spectroscopy in skeletal muscle: experts' consensus recommendations. NMR Biomed 34:e4246
111. Li R, Xiao HF, Lyu JH et al (2017) Differential diagnosis of mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) and ischemic stroke using 3D pseudocontinuous arterial spin labeling. J Magn Reson Imaging 45:199–206. https://doi.org/10.1002/jmri.25354
112. Oppenheim C, Galanaud D, Samson Y et al (2000) Can diVusion weighted magnetic resonance imaging help diVerentiate stroke from stroke-like events in MELAS? J Neurol Neurosurg Psychiatry 69(2):248–50
113. Sakai Y, Kira R, Torisu H, Ihara K, Yoshiura T, Hara T (2006) Persistent diffusion abnormalities in the brain stem of threechildren with mitochondrial diseases. AJNR Am J Neuroradiol 27(9):1924-1926
114. De Picker LJ, Morrens M, Branchi I et al (2023) TSPO PET brain inflammation imaging: a transdiagnostic systematic review and meta-analysis of 156 case-control studies. Brain Behav Immun 113:415–431. https://doi.org/10.1016/j.bbi.2023.07.023
115. Wijesinghe SS, Rowe JB, Mason HD et al (2025) Post-mortem validation of in vivo TSPO PET as a microglial biomarker. Brain 148:1904–1910. https://doi.org/10.1093/brain/awaf078
116. Van Den Ameele J, Hong YT, Manavaki R et al (2021) PK11195-PET brain imaging of the mitochondrial translocator protein in mitochondrial disease. Neurology 96:E2761–E2773. https://doi.org/10.1212/WNL.0000000000012033
117. Liu F, Ruan W, Wang Y, Lan X (2019) Simultaneous 18 F-FDG PET/MRI assists diagnosis of a rare disease, MELAS. Clin Nucl Med 44:81–82. https://doi.org/10.1097/RLU.0000000000002344
118. Ikawa M, Okazawa H, Arakawa K et al (2009) PET imaging of redox and energy states in stroke-like episodes of MELAS.

Mitochondrion 9:144–148. https://doi.org/10.1016/j.mito.2009.01.011
119. Yoder KK, Nho K, Risacher SL et al (2013) Influence of TSPO genotype on 11C-PBR28 standardized uptake values. J Nucl Med 54:1320–1322. https://doi.org/10.2967/jnumed.112.118885
120. Azimi MS, Rahmim A, Arabi H et al (2025) Critical review of partial volume correction methods in PET and SPECT imaging: benefits, pitfalls, challenges, and future outlook. Eur J Nucl Med Mol Imaging. https://doi.org/10.1007/s00259-025-07612-5
121. Kandemirli SG, Al-Dasuqi K, Aslan B et al (2025) Overview of neuroimaging in primary mitochondrial disorders. Pediatr Radiol 55:765–791
122. Bellanti R, Keh RYS, Keddie S et al (2025) Plasma periaxin is a biomarker of peripheral nerve demyelination. Brain. https://doi.org/10.1093/brain/awaf234
123. Keddie S, Smyth D, Keh RYS et al (2023) Peripherin is a biomarker of axonal damage in peripheral nervous system disease. Brain 146:4562–4573. https://doi.org/10.1093/brain/awad234
124. Orssaud C, Reynier P (2025) MetabOCT: a clinical trial looking for a metabolomic signature predicting the onset of Leber's hereditary optic neuropathy in healthy MtDNA mutations carriers. Metabolomics. https://doi.org/10.1007/s11306-025-02328-x
125. Raman S, Chentouf L, DeVile C et al (2018) Near infrared spectroscopy with a vascular occlusion test as a biomarker in children with mitochondrial and other neuro-genetic disorders. PLoS ONE. https://doi.org/10.1371/journal.pone.0199756
126. Khaksari K, Chen WL, Chanvanichtrakool M et al (2024) Applications of near-infrared spectroscopy in epilepsy, with a focus on mitochondrial disorders. Neurotherapeutics 21:e00323
127. Saito S, Takahashi Y, Ohki A et al (2019) Early detection of elevated lactate levels in a mitochondrial disease model using chemical exchange saturation transfer (CEST) and magnetic resonance spectroscopy (MRS) with 7T MR imaging. Radiol Phys Technol 12:232–233
128. Khan AS, Peterson KA, Vittay OI et al (2024) Deuterium metabolic imaging of Alzheimer Disease at 3-T magnetic field strength: a pilot case-control study. Radiology. https://doi.org/10.1148/radiol.232407
129. Ahmadian N, Gosselink M, Otto S et al (2025) Dynamic deuterium metabolic imaging in glioblastoma at 7T. Magn Reson Mater Phys Biol Med. https://doi.org/10.1007/s10334-025-01299-3
130. De Feyter HM, de Graaf RA (2021) Deuterium metabolic imaging – back to the future. J Magn Reson. https://doi.org/10.1016/j.jmr.2021.106932
131. Tsukada H, Kanazawa M, Ohba H et al (2016) PET imaging of mitochondrial complex i with $^{18}$F-BCPP-EF in the brains of MPTP-treated monkeys. J Nucl Med 57:950–953. https://doi.org/10.2967/jnumed.115.169615
132. Engle J, Saberi P, Bain P et al (2024) Oxygen extraction fraction (OEF) values and applications in neurological diseases. Neurol Sci 45:3007–3020
133. Engle J, Saberi P, Bain P, Ikram A, Selim M, Soman S (2024) Oxygen extraction fraction (OEF) values and applicationsin neurological diseases. Neurol Sci 45(7):3007-3020. https://doi.org/10.1007/s10072-024-07362-6
134. Wylezinska-Arridge M, Pizzamiglio C, Küppers F, Shah NJ, Mancini L, Thomas D, Pitceathly RDS, Thornton JS (2025) Calfmuscle oxygen extraction fraction measurement using the multi contrast EPI with keyhole sequence – feasibilitystudy. In: Proceedings of the international society for magnetic resonance in medicine (ISMRM). Honolulu, HI, USA. Abstract 2476. Available from: https://archive.ismrm.org/2025/2476.html
135. Tan L, Zschüntzsch J, Meyer S et al (2024) Non-invasive optoacoustic imaging of glycogen-storage and muscle degeneration in late-onset Pompe disease. Nat Commun. https://doi.org/10.1038/s41467-024-52143-6
136. Xia W, Need E, Schiavone C et al (2025) Image-guided targeting of mitochondrial metabolism sensitizes pediatric malignant rhabdoid tumors to low-dose radiotherapy. Sci Adv 11(21):eadv930
137. Zhang C, Qiu Z, Zhang L et al (2022) Mitochondria-targeted fluorescence/photoacoustic dual-modality imaging probe tailored for visual precise diagnosis of drug-induced liver injury. Anal Chem 94:6251–6260. https://doi.org/10.1021/acs.analchem.2c00041
138. Else TR, Wright L, Schon K, Tiet MY, Seikus C, Ashby E, et al. Photoacoustic imaging in mitochondrial disease. medRxiv.2026:2026.03.10.26347962.https://www.medrxiv.org/content/10.64898/2026.03.10.26347962v1
139. Alix JJP, Plesia M, Stockholm D et al (2024) In vivo Raman spectroscopy of muscle is highly sensitive for detection of healthy muscle and highly specific for detection of disease. Anal Chem 96:15991–15997. https://doi.org/10.1021/acs.analchem.4c03430
140. Alix JJP, Plesia M, Shaw PJ et al (2023) Combining electromyography and Raman spectroscopy: optical EMG. Muscle Nerve 68:464–470. https://doi.org/10.1002/mus.27937